

The pedigree chart illustrates the family history across five generations:

- Generation I:** Consists of an unaffected male (1) and an unaffected female (2).
- Generation II:** Includes an unaffected male (1), an affected female (2) with a value of 0.23, and an unaffected male (3).
- Generation III:** Shows a complex set of relationships with various individuals, including affected males (10, 13) and females (4, 5, 6, 7, 8, 9, 11, 12, 14, 15, 16, 17) with associated numerical values.
- Generation IV:** Features several individuals, including affected males (1, 2) and females (3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23) with numerical values.
- Generation V:** The final generation shown, with individuals 1 through 10, including affected males (1, 2, 3, 4, 5, 6, 7, 8, 9, 10) and females (1, 2, 3, 4, 5, 6, 7, 8, 9, 10) with numerical values.

FIG. 1C



N288D



1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
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FIG. 3A

G T A T G G A A C A G G A T G T T T C T T A C T A T G T <sup>A</sup>/<sub>G</sub> A T A C A G G C C A T A A G

270

310

GK N288D Mutant

- glpk\_human
- glpk\_rat
- glpk\_mouse
- glpk\_ecoli
- glpk\_pseae
- glpk\_entca
- glpk\_haeln
- glpk\_bacsu
- glpk\_yeast
- glpk\_mycge
- glpk\_enlfa
- glpk\_mycpn
- glpk\_syny3

FQIGQAKNTYGTGCFLLDITGHIKCVFSDHGLIITTVAYKIGR SEQ ID NO: 6  
 FQIGQAKNTYGTGCFLLDITGHIKCVFSDHGLIITTVAYKIGR SEQ ID NO: 7  
 FQDGGQAKNTYGTGCFLLDITGHIKCVFSEHGLIITTVAYKIGR SEQ ID NO: 8  
 FQDGGQAKNTYGTGCFLLDITGHIKCVFSEHGLIITTVAYKIGR SEQ ID NO: 9  
 VKFGMAKNTYGTGCFMIMNTGKAVKSENGLIITTIAC--GP SEQ ID NO: 10  
 VF:PGQAKNTYGTGCFMIMNTGDKAVKSTHGLIITTIAC--GP SEQ ID NO: 11  
 FEKGMINKNTYGTGAFIVMNTGEEFQIISDNDLLTITGY--GI SEQ ID NO: 12  
 VHIAGQAKNTYGTGCFMIMNTGKAVKSENGLIITTIACNAKG SEQ ID NO: 13  
 FEFGMGKNTYGTGCFMIMNTGKATKSEHGLIITTIAC--GI SEQ ID NO: 14  
 YKPGAACKTYGTGCFLLDITGHIKCVFSEHGLIITTVAYKIGR SEQ ID NO: 15  
 TFE:PGMVKNTYGTGCFVIMNIGDKPTLSKHNIITTVAWQIEN SEQ ID NO: 16  
 FE:PGMVKNTYGTGCFVIMNIGDKPTLSKHNIITTVAWQIEN SEQ ID NO: 17  
 VE:PAVMKNTYGTGCFMIMNIGNEIKYSQINLIITTVAWQLEN SEQ ID NO: 18  
 DRPGLLKCTYGTGAFLVNTGQIVTRSQHRIITSTVAVWTQIN SEQ ID NO: 19

FIG. 3B

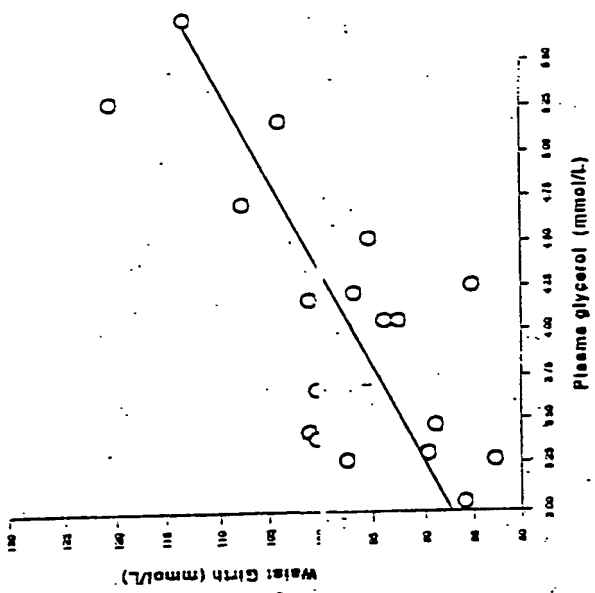


FIG. 4A

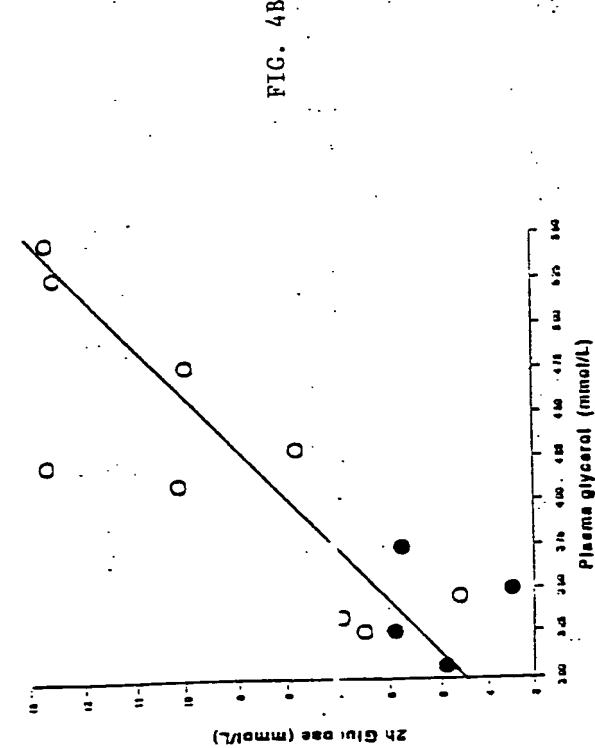


FIG. 4B

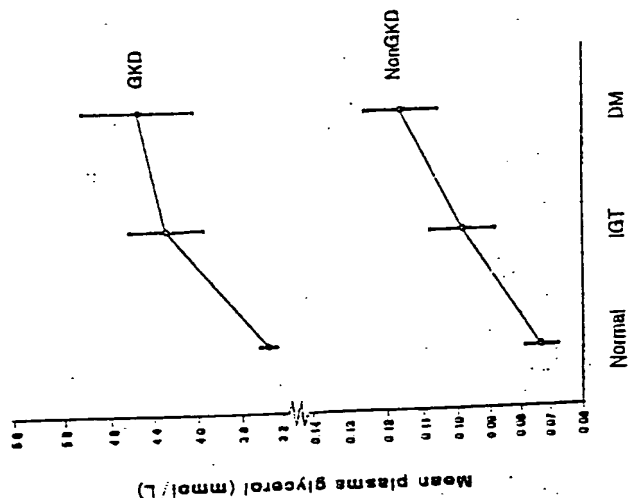
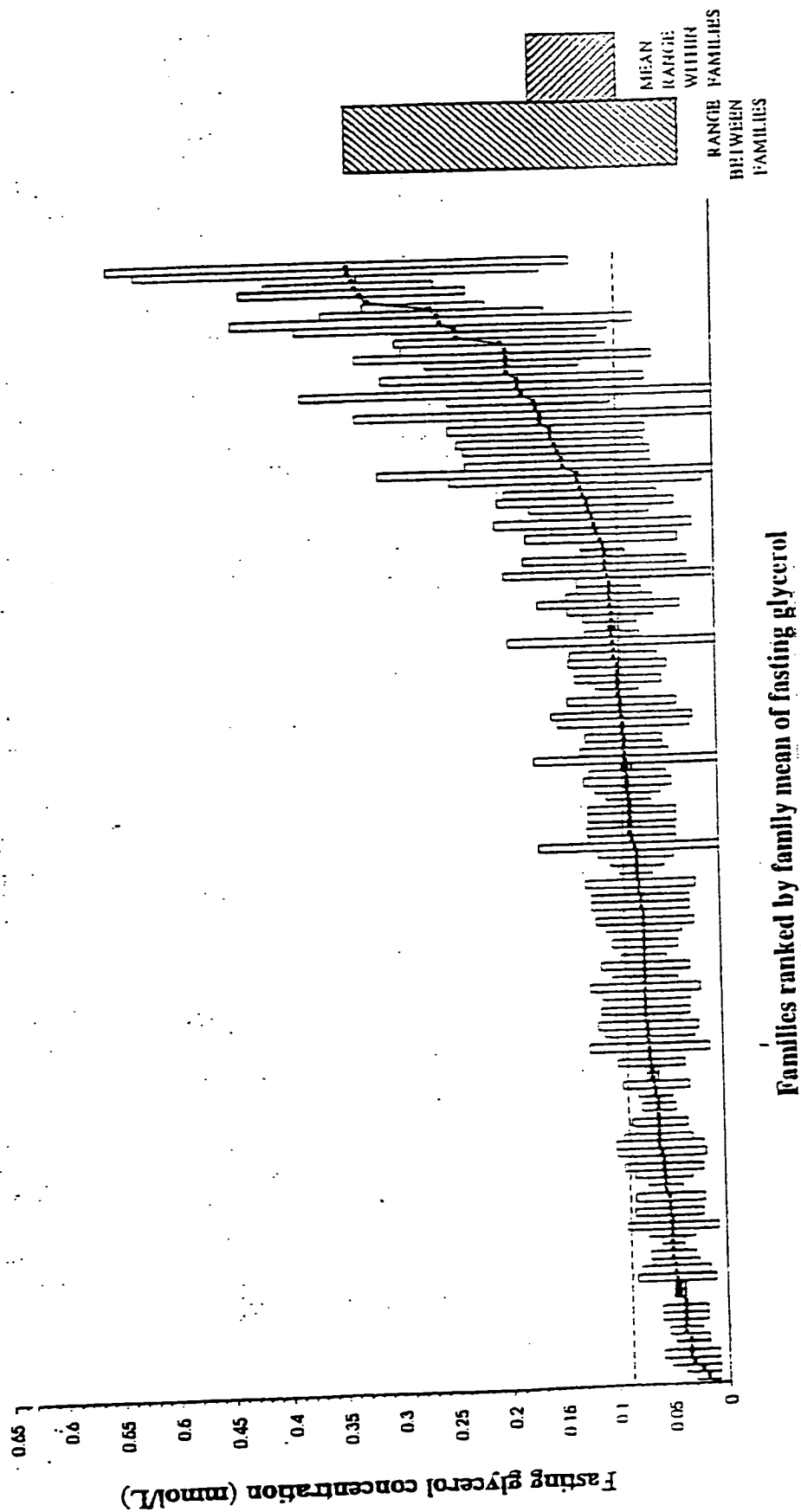


FIG. 4C

FIG. 5



poly: A/G  
location: 13th base of exon 3

ATGCCTTCTTTTGTCAAAGATGGGTGGAACA [A/G] GACCCTAAGGAAATTCTACAT  
TCTGTCT SEQ ID NO: 1

CAA vs CAG ==> silent

poly: A/C  
location: 17th base of intron 8  
TAATGGTAAAAACAAACAAA [A/C] AAACAAAAACACACCAAAAAACCAA

SEQ ID NO: 2

poly: A/G  
location: 29th base of exon 10

TTCATTCTCCCTTCAACCATAGGTATGGAACAGGATGTTTCTTACTATGT [A/G] AT  
ACAGGCCATAAGGTtGGTTTTTAATAAAAATGATTAAGTCA SEQ ID NO: 3

AAT vs GAT ==> N to D

poly: G/T  
location: 22nd base of intron 12  
GAAATTGGTGAGTGTGTTCTAACAAAAG [G/T] TTAGAAAATCTGAAAAATGACACA  
TTTCA SEQ ID NO: 4

FIG. 6

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SEQ ID NO: 5

Exon 1:  
GGTTCAGCGGACGCGCGCGGCCCTCGGTCTCTGGACTCGTCACCTGCCCCCTCCCCCTCCCGCC  
GCCGTCACCCAGGAAACCGGCCGCAATCGCCGGCCGACCTGAAGCTGGTTTCATGGCAGCCT  
CAAAGAAGGCAGTTTTGGGGCCATTGGTGGGGGCGGTGGACCAGGGCACCAGTTCGACGCGC  
TTTTTGCTGAGCCCGGGGTGACATGTGAAGAGGCGCTGAGC

Exon 2:  
TGTAACGACGGCCAGTCATCCTTGATATCTGCCTGCATTTTTACATTAATATTACAATAT  
CTTTTTCAGGTTTCAATTCAAAAACAGCTGAACTACTTAGTCATCATCAAGTAGAAATAAA  
ACAAGAGTTCCCAAGAGAAGGGTATGTTTCCTAATTTAATATGTAAAGACACATTATGTTTG  
TTAGTCCATCTCACCCAACCTTGCCC

Exon 3:  
CAATGCCTTCTTTTGTCAAAGATGGGTGGAACA [A/G] GACCCTAAGGAAATTCTACATTCT  
GTCTATGAGTGTATAGAGAAAACATGTGAGAACTGGACAGCTCAATATTGATATTTCCAA  
CATAAAAGGTATTTTAGTAGAATATTTTACCCACA

Exon 4:  
TGTAACGACGGCCAGTTGAGAGCTGTTTTCTGAAGTAGTTCCTACTTGTTAAATTTTTG  
ACTTCCTTCTGTTTAACTTTCTCTTTAAAGCTATTGGTGTGAGCAACCAGAGGAAACCACT  
GTAGTCTGGGACAAGATAACTGGAGAGCCTCTCTACAATGCTGTGGGTAAAGCTGTCATGCAT  
GGATGTCAAATGTAGGGCCTTTCTTCACATTGCAA

Exon 5:  
TGTAACGACGGCCAGTTCCCTTGATAGTGATTTTCAGTAAGTTCTTATTTTTTTAAATGAAG  
TTTTTCATGTATATTATTTTATTTTGGTCTATAGTGTGGCTTGATCTAAGAACCCAGTCTAC  
CGTTGAGAGTCTTAGTAAAAGAATTCAGGAAATAATAACTTTGTCAAGGTAAGAATTTCTT  
CAGAAGTATACTATAAGAATGTTTCTTTTTTTAAAAAAGTTTGCAGATTTCACTAGAAAGA  
AGCATCTTATGGTACAATAGTTATTTGATACAATTTATAGAATCTTTTTCCCGGATAATTGA  
GGCC

Exon 6:  
TGTAACGACGGCCAGTTTCTTTTGTGTTGGTGGTTTTGTTTTAACTGTTACACTTTTCAT  
TTGCTAACTGAACTTCACAACCTGCTTTTAGTCCAAGACAGGCCTTCCACTTAGCACTTACTT  
CAGTGCAGTGAACTTCGTTGGCTCCTTGACAATGTGAGAAAAGTTCAAAAGGCCGTTGAAG  
AAAAACGAGCTCTTTTTGGGACTATTGATTCATGGCTTATTTGGGTATGTTTAAATATAATG  
GATATATGGAGAAATTTTTTCAGAAATTTTTCTAGACTGCCTTGCTTATTGTTTCTACTAGC  
AGGTCAGACTTTTTAATTAGCA

FIG. 7A

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Exon 7:

TGTAACGACGGCCAGTTGTGCTCTGCTGATTATGACCCCTTAACAATATGTAAATTAAATT  
GCCAATAAGTACAAATTTAACCTGATTTTTTTTACTCTGCCTAGAGTTTGACAGGAGGAGTCA  
ATGGAGGTGTCCACTGTACAGATGTAACAAATGCAAGTAGGACTATGCTTTTCAACATTCAT  
TCTTTGGAATGGGATAAACAACTCTGCGAGTAAGTTCTGTTTTGCTCTAAATATAGTTTTCC  
CAATACACTACCTATTTATAACCGAAATCTTAATATTTTCAGATGTCAGTGGAGCA

Exon 8:

TGTAACGACGGCCAGTACAGTGTTAAATACCCAATCTTCTTGTTCAGATTTTTTGGGA  
ATTCCAATGGAAATTTCTTCAAATGTCCGGAGTTCTTCTGAGATCTATGGCCTAATGGTAAA  
AAACAAACAAA [A/C] AAACAAAAACACACCAAAAAACAAAAACAAAAAAACC  
TAATAATTAAAGTTTTTTTATTACAAACAAGTTTACTATTTCATAATTCAAAGTCAACTGT  
GTTATGTTTTGTGACTTAAAACTTTACAGTCCTTTTTTACAATGG

Exons 9A and 9B

AAAGCTGGGGCCTTGGAAGGTGTGCCAATATCTGGGGTAAGTTTCATCACCAAGTGTCTCCC  
CATCCCCACCCTTCCCCATGTTATGGCTTTCCCTCCTCTTAGTTCATCAGTGTGCCTCTTTTT  
AACTAGGGAAAAACAAGTAAAAGTTGCAAAATGGANNNNTCTTGTTCTTACATGTCATACT  
GTGGGCCATTGAGAATCTTTTGAATAAATTAATTTTAACTCTCCCTTCCCATACTATTATC  
TTACATATTAACAAATGGTATTAACAAATGGGAAAAATGGCCAAATGGAGAAAATGCAAGGA  
AATAGACAGTTCATTCTTTGATAAAATAAAAAATGAAAAATAAATCCTATGGCTCTTCTAAAA  
AGAAAGTTAATACTATTGTATTAGTCAGTGTCTTTATTGTCAATTTATACTTTTCAGTGTTTA  
GGGACCAGTCTGCTGCATTGGTGGGACAAATGTGCTTCCAGATTGGACAAGCCAAAAATAC  
GTGAGTTTAAAGAAACAGACTTAAAAACCAATGCTGTTTTGTTTTTTCTACTTGGTGCTTTGA  
ATAAGGAAAAGCTTTTGAAGTTCATCCAGGATGAAAATCAATAGCTTAATAGCTCCAATATG  
CATATATACACTTTTTTACCATTTTTTTTATATCTTTAAATAAAATACAAA  
TGCCATATATATGCACACTGATGAAGCTTATAAAGACCTAAATTTGTAGGCTGGGCGCGG

Exons 10 and 11:

TTATTTGCTTTCAATAAAATTGTCTTCTATTTCATTCTCCCTTCAACCATAGGTATGGAACAG  
GATGTTTCTTACTATGT [A/G] ATACAGGCCATAAGGTGGTTTTTTTAAATTAAAAAATTGA  
TTTAAAAGTCTAAGTTCATCTAAATAATGCTTGAACATAATTTACTATTAAACAACTTTTAG  
TCTTTAGCTTTTACTTAATCTTTATCAGGGTTTAAATTTAGAGCTCAATACAAAATTTGAATC  
GTTCTAATAAGAACCATTTTAGACTCTTTGAATTTTATATGTGTGTTTTTAAATTGTGCTGGG  
GGGAAATCTAGACTGAGACCTCATCAAATCTTAATGCAAATCTAATTTGAAACAAGGAATA  
AACTTTTTTATACAGCTTAAATGTGTTCTTAATCTGATCGTTTTGACTGTAAGGATTTATTT  
TAAAAATTGGTTTATTGATTGCATTATTTTGTACCTATGTTATTTTAACTTTAAAAAAAAGT  
TCTCATGTTATCTTTTCATTTTCCACTACTGAAATCTTTTTTTTTTTCTTTCTTACAGTGTGT  
ATTTTCTGATCATGGCCTTCTCACCACAGTGGCTTACAACTTGGCAGAGACAAACCAGTAT  
ATTATGCTTTGGAAGTAAGTTCTTTTTTAATCAATATGGATAATATGACAAACATTCAAAGCT  
AATAAAAATCACAGAGTTTTCTAACACTTTTCTGGTAAATCTTAATACAGAGGACTCAAAAA  
GTTCTGCTTTCTTGGCATTGATTGAGTTGAAGGAACCTGAACTGATCTGGGTGTCAGGAC  
TCACAGGAGACCTTGATTAGATTGGTTCCTCAGTTCTTATGCCAATTAATCATGTACCTTA  
GGCATATTACTTGAGAGCTCTACAATGTGAGGTTTTTTTTTTTTTTTATCTCTAAAGTTTAAAT  
CGGATTAACGTGCTCTCTAACATTTCTTTCATCTTGAAAATCTTTGATTTTATAAATAAAA  
TGCTCCAGTGTTCAAAGAGAACCTGGGCACAAATAGGCAGAACAACTCTCTTCACTTGTC  
TCCTCATAAAAAATAAATTTTGTGTAACATTTTGATATAGAAAAGAAAGCGACGAGATTTATG  
CCACTTATCACTGGAAACATTTGTTTTCAAACATTTTGTATGTTATAGTAGGAATATGCCAG  
CCTAAGCCTATA

FIG. 7B

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000201-10208

Exon 13:

Exons 14 and 15:

Exon 16:

Exon 17:

FIG. 7C

Exon 18:

TGTAACGACGGCCAGTTGGTTTGGTTTGCTTGACTGGAATCTCTTCTGCTTGGATGACCA  
CAGGTGACCCTAGTATCTTCTGTAGTCTGCCCTTGGGCTTTTTTATAGTGAGTAGCATGGTA  
ATGTTAATCGGAGCAAGGTACATCTCAGGTAGTTACTCTTTAAATTAGACAACTCTATTAG  
TTAGCTTTAATGTTTTCGTGTATAACTTAGCAGAAATTTTTCAGTGTTCATTCTTTCTG  
TGTCTAGGAAGCTGGAAAATCAATTAAAGGTCTAATTAGTTAGACCAATTAATCTTTGGGGG  
CAGTTAGAAGTAAGAAGTGTGACTCTGCTTACCCTTTTTAAATTTTAAATGTGATGACTTCT  
TTAAGAGGGACTACATTCTGCTGTGAGCTGCAGCAATAAGCAAAAGTGAAAATACTAATATT  
TAAATGACAGGACTTTTCACTGACTGCTGAAAGTTAAAGTATACTT

Exon 19:

AAAATTACTGGCTTAAATGGAAATGATGCTTCTTATTCTGTATGTTCCCATGAAAGTGAAAC  
TTAAAAAAAATTTCATGATTAGGGTTTCATGAAAAGGCCCTTGTCTTCTATGAAAATTGAGAC  
AGGTTGCATCTCTCTAAGCTAAAAGATGGGCTATGTGTCTAGAGTCTTAGACTTCTAAAATG  
CATGTGGTCACTATATGTAGGTTATCTCTTCGGTGACATACTGCAATTTGAGAGGGCTGG  
AAATTGTTTGCCTTGGTAAACGATTAGCAACAGTGGCAATATTTGTTAATTTTGGAAATTGGC  
CCTGTTTGTGCAATTTAATTGTGAGGCATGATTTAGAAATCATATGGACTTTCTAGCTTAA  
TAAATGATTGAATCATCTGCATTGCTTTAACTCCTGAATTGTATGCATGTATTATTGACATA  
TATGGTTTTTGTTCCTTCAGGTATTCCATAAAACCTACCAACTCATGGATTCCCAAGA  
TGTGAGCTTTTACATAATGAAAGAACCAGCAATTCTGTCTCTTAATGCAATGACACTATT  
CATAGACTTTGATTTTATTTATAAGCCACTTGCTGCATGACCCTCCAAGTAGACCTGTGGCT  
TAAAATAAAGAAAATGCAGCAAAAAGAATGCTATAGAAATATTTGGTGGTTTTTTTTTTTTT  
TAAACATCCACAGTTAAGGTTGGGCCAGCTACCTTTGGGGCTGACCCCTCCATTGCCATAA  
CATCCTGCTCCATTCCCTCTAAGATGTAGGAAGAATTCGGATCCTTACCATTGGAATCTTCC  
ATCGAACATACTCAAACACTTTTGGACCAGGATTTGAGTCTCTGCATGACATATACTTGATT  
AAAAGGTTATTACTAACCTGTTAAAAATCAGCAGCTCTTTGCTTTTAAAGAGACACCCTAAAA  
GTCTTCTTTTCTACATAGTTGAAGACAGCAACATCTTCACTGAATGTTTGAATAGAAACCTC  
TACTAAATTATTAATAAGACATTTAGTGTTCTCACAGCTTGGATATTTTCTGAAAAGTTA  
TTTGCCAAAAGTGAATCCTTCAGATGTTTTCCATGGTCCCACTAATTATAATGACTTTCTG  
TCTGGGTCTTATAGGAAAAGATACTTTCTTTTTTCTTCCATCTTTCCTTTTTATATTTTAA  
CTTTGTATGTATAACATACATGCCTATATTTTATACACTGAGGGAGCCCATTTATAAATA  
AAGACCACATTATATTCAGAAGGTTCTAACAGGG

FIG. 7D

TABLE 1

Characteristics of carriers of the N288D GK gene mutation  
and of their unaffected relatives

	Men			Women		
	N288D carriers	Unaffected relatives	p	N288D carriers	Unaffected relatives	p
N	18	18		14	14	
Age (years)	46,4±14,2	42,0±18,8	0.32	44,9±13,5	43,7±17,8	0.87
Uncorrected triglyceride (mmol/L) <sup>(1)</sup>	6,26±1,13	2,05±0,54	<0,0001	2,84±1,20	1,30±0,65	0,0002
Glycerol (mmol/L)	3,99±0,71	0,10±0,04	<0,0001	0,54±0,14	0,10±0,02	<0,0001
Corrected triglyceride (mmol/L) <sup>(1)</sup>	2,27±0,75	1,95±0,53	<0,0001	2,31±1,22	1,19±0,67	0,03
Free fatty acid (mmol/L)	0,77±0,22	0,57±0,25	0,01	1,29±0,35	0,76±0,17	0,0004
Fasting glucose (mmol/L)	5,2±0,74	4,8±0,31	0,13	5,0±0,7	4,6±0,3	0,10
2h glucose following OGTT (mmol/L)	7,9±3,1	5,8±1,6	0,02	7,0±5,1	5,0±2,1	0,04
Fasting insulin (mU/L) <sup>(1)</sup>	13,3±14,0	15,1±14,8	0,62	12,2±13,1	9,0±3,4	0,60
Waist girth (cm)	97,7±9,3	88,1±12,3	0,01	88,5±3,3	79,3±5,3	0,03
Body mass index (kg/m <sup>2</sup> )	27,9±4,1	24,9±3,9	0,03	28,1±5,5	23,1±2,3	0,001
% total body fat	27,1±7,2	22,9±7,6	0,01	46,3±3,1	33,9±11,3	0,001

(1) geometric mean, p value after log transformation

Figure 8

TABLE 2

Fasting plasma glycerol concentration (mmol/L) in the initial cohort of 1056 individuals,  
by risk factor of glucose intolerance and diabetes mellitus

		No.	Glycerol geometric mean $\pm$ SD	p
Gender	men	717	0.065 $\pm$ 0.081	<0.0001
	women - premenopausal	137	0.071 $\pm$ 0.093	
	- menopausal	202	0.099 $\pm$ 0.085	
Age (y)	<50	486	0.071 $\pm$ 0.082	0.0015
	50 - 60	408	0.076 $\pm$ 0.106	
	>60	163	0.083 $\pm$ 0.053	
Fasting glucose (mmol/L)	< 5.2	449	0.063 $\pm$ 0.080	<0.0001
	5.2 - 5.9	336	0.070 $\pm$ 0.090	
	6.0 - 6.9	271	0.090 $\pm$ 0.100	
Fasting insulin (UI)	<15	637	0.067 $\pm$ 0.082	0.02
	$\geq 15$	419	0.086 $\pm$ 0.101	
2 hours glucose (mmol/L)	<7.3	572	0.062 $\pm$ 0.071	<0.0001
	7.3 - 11.0	283	0.081 $\pm$ 0.101	
	$\geq 11.1$	201	0.102 $\pm$ 0.110	
Triglyceride (mmol/L)	$\leq 2.2$	389	0.057 $\pm$ 0.062	<0.0001
	>2.2	667	0.082 $\pm$ 0.103	
Free fatty acid (mmol/L)	<0.6	589	0.066 $\pm$ 0.054	<0.0001
	$\geq 0.6$	467	0.111 $\pm$ 0.112	
Body mass index (kg/m <sup>2</sup> )	$\leq 27$	423	0.060 $\pm$ 0.087	<0.0001
	>27	623	0.079 $\pm$ 0.097	

p value from one-way ANOVA

Figure 9

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TABLE 3. Multivariate analysis of the relationships of fasting plasma glycerol concentration with impaired glucose tolerance (2h glucose 7.3-11.0 mmol/L following a 75 g oral load) before and after adjustment for covariates identified in

	Model 1	Model 2	Model 3	Model 4
<b>Glycerol (log)</b>				
β	1.75	1.62	1.46	0.77
Odds ratio	5.76	5.42	4.33	2.41
p	<0.0001	<0.0001	<0.0001	0.01
<b>Triglyceride (log)</b>				
β		0.54	0.35	0.11
Odds ratio		1.75	1.42	1.11
p		0.02	0.11	0.63
<b>Body mass index (kg/m<sup>2</sup>)</b>				
β			0.10	0.03
Odds ratio			1.10	1.03
p			<0.0001	0.01
<b>Fasting insulin (log)</b>				
β				0.37
Odds ratio				1.31
p				0.39
<b>Fasting glucose (mmol/L)</b>				
β				1.13
Odds ratio				2.65
p				<0.0001
<b>Free fatty acid (log)</b>				
β				1.12
Odds ratio				4.13
p				0.007

Odds ratios are expressed as the increase in the risk of 2h glucose ≥7.3 mmol/L following a 75 g oral charge, associated with a 1-SD increase in the variables studied. β denotes the standardized estimate which is the parameter estimate of each variable in the multivariate logistic model. All models included age and gender as covariates. Otherwise, only the variables included in each model are shown. Subjects with severe hypertriglyceridemia due to the N788D mutation in the

Figure 10

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